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Hot and cold fibrosis: The role of serum biomarkers to assess immune mechanisms and ECM-cell interactions in human fibrosis

Andressa de Zawadzki[1,*], Diana J. Leeming[1], Arun J. Sanyal[2], Quentin M. Anstee[3,4], Jörn M. Schattenberg[5], Scott L. Friedman[6], Detlef Schuppan[7,8,†], Morten A. Karsdal[1,†]

Summary

Fibrosis is a pathological condition characterised by excessive accumulation of extracellular matrix (ECM) components, particularly collagens, leading to tissue scarring and organ dysfunction. In fibrosis, an imbalance between collagen synthesis (fibrogenesis) and degradation (fibrolysis) results in the deposition of fibrillar collagens disrupting the structural integrity of the ECM and, consequently, tissue architecture. Fibrosis is associated with a wide range of chronic diseases, including cirrhosis, kidney fibrosis, pulmonary fibrosis, and autoimmune diseases. Recently, the concept of "hot" and "cold" fibrosis has emerged, referring to the immune status within fibrotic tissues and the nature of fibrogenic signalling. Hot fibrosis is characterised by active immune cell infiltration and inflammation, while cold fibrosis is associated with auto- and paracrine myofibroblast activation, immune cell exclusion and quiescence. In this article, we explore the relationship between hot and cold fibrosis, the role of various types of collagens and their biologically active fragments in modulating the immune system, and how serological ECM biomarkers can help improve our understanding of the disease-relevant interactions between immune and mesenchymal cells in fibrotic tissues. Additionally, we draw lessons from immuno-oncology research in solid tumours to shed light on potential strategies for fibrosis treatment and highlight the advantage of having a “hot fibrotic environment” to treat fibrosis by enhancing collagen degradation through modulation of the immune system.

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Introduction

Fibrosis describes a continuous scaring process characterised by an excessive accumulation of extracellular matrix (ECM) components in response to stimuli connected to injury and chronic inflammation in the course of various diseases, including metabolic, vascular and autoimmune diseases like systemic sclerosis. In healthy conditions, ECM homeostasis is governed by a balance between the rates of fibrogenesis and fibrolysis (*i.e.* the formation and degradation, respectively) of fibrous macromolecules that provide structural support and perform numerous biological functions in multicellular tissues. Herein, we focus on collagens, which are not only the most abundant structural proteins of the ECM, but also key molecules that direct the architecture of complex organ systems, maintain organ homeostasis, and orchestrate multicellular responses to injury.[1–3]

In chronic inflammatory-fibrotic diseases and in most cancers, *i.e.* in ”wounds that do not heal”,[4,5] ECM homeostasis is disrupted by abnormal rates of formation and degradation, leading to excessive or atypical collagen deposition, a progressive destruction of tissue architecture and a resultant loss of organ function.[2,6] Fibrosis involves a combination of multiple fibrogenic and inflammatory pathways and such complexity yields different disease profiles, which can be represented by different endotypes based on the disrupted balance of the ECM, as demonstrated in the literature through collagen-based biomarkers (Fig. 1).[7,8] Collagens are highly impacted by the chronic disruption of this balance and are the source of serological biomarkers used to assess this process.[9] During fibrosis, the immune landscape within the affected tissues is significantly influenced by ECM remodelling and vice versa, disrupting the complex environment that provides structural and biochemical support to surrounding cells. Immune cells play a pivotal role in fibrosis, either paving the way for fibrogenic remodelling or for homeostatic reorganisation of the ECM in fibrosis resolution.[10–14]

The vast literature on proposed therapies for fibrosis has focused largely on reducing fibrogenesis by targeting cell types or signalling pathways associated with excessive ECM formation.[15,16] However, there is a gap in understanding how the

* Corresponding author. Address: Herlev Hovedgade 207, 2730 Herlev, Denmark; Tel.: +45 4452 5252.
E-mail address: anza@nordicbio.com (A. de Zawadzki).
† Co-senior authors
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Keypoints

- Fibrosis results from persistent or repetitive injuries that lead to chronic inflammation and impaired tissue repair.
- "Hot & cold fibrosis" concept defines the immune landscape within fibrotic conditions based on the presence (hot) or absence (cold) of immune cells.
- The complex interplay between the immune system and extracellular matrix drives fibrosis development and progression.
- Extracellular matrix biomarkers provide promising tools to assess fibro-inflammatory processes in hepatic diseases and to identify disease phenotypes associated with hot or cold fibrosis.
- The concept of hot and cold fibrosis may help to stratify patients in terms of pathophysiological drivers and clinical risk, who otherwise have indistinguishable histological severity.
- Defining fibrosis as hot or cold may aid in designing novel therapeutical strategies to simultaneously intercept fibrogenesis and stimulate fibrosis resolution via the immune system.

degradation of fibrotic tissue becomes insufficient to match ECM production and how to stimulate the immune system to activate cellular mechanisms or produce enzymes that resolve/degrade the excessive fibrotic tissue. Understanding these interactions between the ECM and immune system will provide insights for future therapies to prevent and/or treat fibrosis by inducing its regression from two different standpoints: inhibiting fibrosis formation and, perhaps more importantly, enhancing fibrosis resolution/ECM degradation[9,16–21] to regain a healthy equilibrium between fibrogenesis and fibrolysis.

The medical and scientific communities generally describe fibrosis according to the underlying condition or tissue affected (*e.g.*, liver fibrosis, pulmonary fibrosis) and fibrosis stage (early-stage or advanced fibrosis). The terminology "hot" and "cold" fibrosis has recently emerged in the context of discussing the degree of inflammation or activity associated with fibrotic conditions, similar to the concept of "hot and 'cold' tumours" which has been widely used in oncology to describe the immunological microenvironment which is pivotal in determining cancer progression or cancer control.[6,22,23] This concept has also been validated in the heart, establishing it as a more generalised feature of tissue responses to injury that is not restricted to the liver.[24]

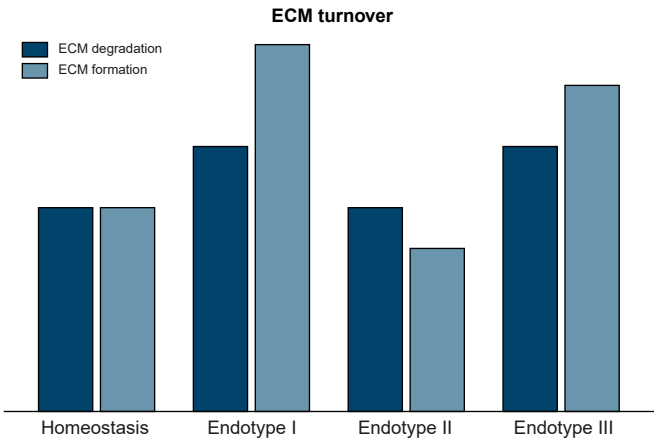

Fig. 1. ECM turnover as represented by the product between rates of formation and degradation (y-axis) of ECM components. ECM turnover is an essential process for homeostasis maintenance. Fibrosis/fibrogenesis results from the disruption of ECM balance due to excessive formation and/or deficient degradation of ECM components that can occur in different settings and different rates in which fibrogenesis overtakes fibrolysis. Three different endotypes representing fibrogenesis process: (i) endotype I - fibrogenesis endotype: significant increase in ECM formation with same level of degradation of homeostasis; (ii) endotype II – fibrosis resolution endotype: decrease in ECM formation with the same level of ECM formation; (iii) endotype III – fibrosis with elevated tissue turnover: significant increase in fibrogenesis and fibrolysis with abnormal elevated rates of matrix formation and degradation. The representation of these phenotypes is inspired by data that has been described by Karsdal *et al.*, 2023[7] and Pehrsson *et al.*, 2022[8] displaying how the balance between formation and degradation of ECM can be assessed from collagen biomarkers. ECM, extracellular matrix.

Applying the concept of "hot" and "cold" to fibrosis is key not only for phenotyping different fibrotic environments, but also for predicting the reversibility and treatability of fibrosis, including the design of strategies that engage the immune system. In this article, we outline the dynamics of hot and cold fibrosis and the impact of collagens on immune system regulation, as well as describing novel ECM/collagen biomarkers that have begun to contribute to a better understanding of the immune landscape behind fibrotic conditions and to serve as biomarkers of antifibrotic responses. It includes insights from immuno-oncology studies in solid tumours that highlight potential strategies for treating cancer as well as fibrosis. Finally, ECM biomarkers promise not only to guide antifibrotic therapy development, but also to enable personalised monitoring of the efficacy of such therapies.

Immune cells in pathogenesis of fibrosis

To understand the differences between hot and cold fibrosis, it is necessary to describe the origins and development of fibrosis in the context of healing and immune status, see Figs 1 and 2. Fibrosis originates from tissue injury or chronic inflammation, which can be caused by bacterial and viral infections, toxins, metabolic derangement, autoimmune reactions, or mechanical injuries. Usually, epithelial or endothelial cells in the tissue that is damaged by the insult secrete or induce danger molecules, cytokines and chemokines. This is followed by the release of proteolytic enzymes, prominently by macrophages, that degrade the subcellular and microvascular basement membrane to provide immune cells access to the injured site. The basement membrane consists of a scaffold composed of a network of type IV collagen that, in conjunction with several

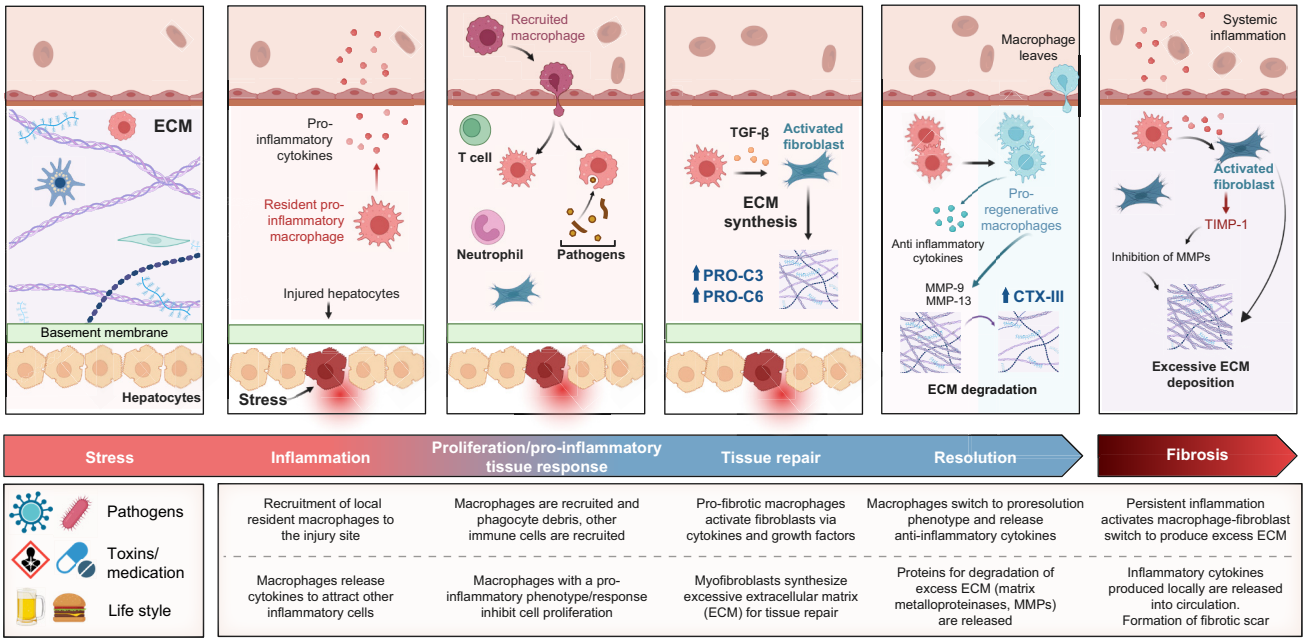

Fig. 2. The mechanisms behind fibrosis pathogenesis highlighting the interplay between inflammation and ECM components. Immune cells of relevance for tissue repair induce both formation and degradation of ECM components which can be monitored via collagen biomarkers or biomarkers reflecting the activation of specific immune cells. Adapted from Zehravi 2022.[214] Figure created with Biorender. ECM, extracellular matrix.

other basement membrane components, functions as a supporting structure for epithelial and endothelial cells and a protective barrier against mechanical stress and uncontrolled access of circulatory cells and certain macromolecules. Along with the degradation of the basement membrane, the early inflammatory processes involve the recruitment of various immune cells including monocytes-macrophages and later T and B cells to the injured site.[25,26] Monocytes-macrophages are among the first responders to tissue injury, and are attracted by epithelial or endothelial mediators, damage-associated molecular patterns, chemokines and cytokines. They release ECM-degrading proteases as well as T and B cell-specific cytokines and chemokines that induce the second wave of lymphocyte responses to the injury site to amplify the initial innate inflammatory response towards an adaptive, antigen-specific T- and B-cell response. This primary phase of macrophage activity, called the pro-inflammation phase, helps to remove debris, dead cells and pathogens from the damaged area. If this eliminates and terminates the insult early enough, innate inflammation subsides and an anti-inflammatory/repair phase begins.[20,25,27]

During the early stages of the repair phase, macrophages with a pro-inflammatory phenotype secrete signalling molecules such as pro-inflammatory cytokines, transforming growth factor-β (TGF-β) and other pro-fibrotic molecules. TGF-β activates (myo-)fibroblasts which comprise primary cells located in the interstitial space of the ECM and pericytes in micro vessels (*i.e.* hepatic stellate cells [HSCs] in the liver); (myo-)fibroblasts are responsible for the synthesis and maintenance of the homeostatic ECM. Upon activation via TGF-β, the usually quiescent fibroblasts proliferate and change their morphology into myofibroblasts which are more contractile and produce large amounts of ECM, predominantly type I and type III collagens. The excess production of collagenous ECM by these cells restores the integrity and functionality of the damaged tissue, providing guidance and anchorage for epithelial recovery.[6] When inflammation is actively resolved, a pro-resolution phase initiates with anti-inflammatory and antifibrotic signals that induce macrophages to produce matrix metalloproteinases (MMPs) and other proteolytic enzymes in order to remove the excess ECM that was previously produced, restoring tissue homeostasis without significant fibrosis.[22,26,28]

Via this phenotypic shift, macrophages play a multifaceted role in the immune response and tissue repair. For years, macrophages have been conceptually categorised into two distinct phenotypes named M1 (inflammatory) and M2 (anti-inflammatory). However, the M1/M2 classification is outdated and increasingly considered oversimplified as it does not reflect the dynamic context-dependent nature of macrophage activation and thus fails to capture the complexity and far greater macrophage heterogeneity.[29,30] Recent advances in spatially resolved transcriptomics and single-cell RNA sequencing have demonstrated that macrophage phenotypes can be described by a full spectrum model of functional states that exist in a context-dependent manner. These studies identify macrophage subtypes based on gene expression, potentially allowing for the development of novel macrophage biomarkers. The recent literature states that macrophages observed in both homeostatic and injured hepatic tissue are heterogeneous and originate either from liver-resident phagocytes produced in the yolk sac (named Kupffer cells [KCs]), bone marrow-derived monocytes or peritoneal macrophages (for subcapsular parts of the liver).[31–33] It has been demonstrated that the origin of macrophages impacts their ability to respond to recruiting or danger signals and thereby execute specific cellular functions that either aggravate or halt tissue injury.

In the liver, several macrophage populations have been identified through distinct transcriptional and functional profiles. Two main populations of monocyte-derived macrophages (MoMFs) with distinct functions have been characterized: Ly6C^{high} MoMFs are pro-inflammatory and Ly6C^{low} MoMFs are involved in tissue repair.[34] During homeostasis, KCs are the predominant macrophage population, while two other additional populations can be observed: bile duct–associated and subcapsular macrophages.[33] KCs have also been further divided into two populations based on the expression of CD206 and ESAM: KC1(CD206^{low}ESAM^{-}) and KC2 (CD206^{high}ESAM^{+}).[33,35]

TREM2+ macrophages (triggering receptors expressed on myeloid cells, family 2) have also been shown to have subpopulations with varied functions. Trem2 expression occurs in two macrophage populations: monocyte-derived macrophages occupying the Kupffer cell niche (MoKC) and lipid-associated macrophages (LAMs) which are present in liver regions where lipids accumulate, and are relevant for steatosis and liver fibrosis.[31,36] MoKCs were found to be the predominant subpopulation during metabolic dysfunction-associated steatohepatitis (MASH) while LAMs were identified as the main population during MASH regression.[32] TREM2+ macrophages have recently been recognised for suppressing inflammation by inhibiting the production of pro-inflammatory factors in KCs and HSCs, and thus playing a protective role in liver-related diseases.[28] Meanwhile, a unique subtype of fibrogenic scar-associated macrophages, identified by single-cell RNA sequencing as CD9+ TREM2+ macrophages, have been detected in scar regions in the liver and recognised as central players in promoting inflammation and HSC activation, and thereby sustaining the fibrotic environment in advanced fibrosis, especially in cirrhosis.[37] Bile duct-associated macrophages with a LAM phenotype have been shown to be enriched in patients with primary sclerosing cholangitis and to be associated with disease severity, with serum osteopontin (OPN, spp1) acting as a biomarker to monitor the accumulation of macrophages around bile ducts.[36] These examples highlight the importance of understanding macrophage heterogeneity and its contribution to liver disease development and progression in the context of designing future therapies targeting specific macrophage phenotypes.

Other immune cells that play a role in the development and progression of fibrosis are neutrophils, T cells, platelets,[38,39] eosinophils, basophils and mast cells.[40] Similarly to macrophages, neutrophils are among the first responders to reach the injured tissue, participating in the initial stages of inflammation. Excessive and prolonged activation of neutrophils can generate a sustained pro-inflammatory and tissue destructive response that activates fibroblasts to a lesser extent, with a milder contribution to collagen production and scar tissue formation. Activated platelets release profibrogenic cytokines and proteins, including TGF-β1, platelet-derived growth factor-B (PDGF-B) and TIMP-1 (tissue inhibitor of metalloproteinases 1) that interact with monocytes/macrophages or HSCs (PDGF-B). Notably, the anti-platelet drug aspirin attenuates early fibrosis in mice and men, prominently by preventing the release of PDGF-B.[41–43] T cells are central for immune response regulation. There are different subsets of T cells with distinct functions including helper T cells, cytotoxic T cells, memory T cells, γδ T cells and regulatory T cells (Tregs). Some of the T-cell subtypes, including T helper 2 and T helper 17 cells, and innate lymphoid cells type 2 which are related to T cells, are implicated in tissue scarring in all types of fibrosis (pulmonary, hepatic, in the gut).[40,44] They again produce profibrogenic cytokines that can directly activate (myo-)fibroblasts.[45,46] Tregs are crucial for maintaining immune homeostasis by suppressing excessive immune responses. A decrease in Treg activity has been observed in early stage fibrotic diseases, while other (wound healing) Treg subsets promote fibrosis, especially via the production of immune suppressive but pro-fibrotic TGF-β1.[47,48] Besides immune cells, HSCs are major protagonists in fibrosis development; recently, a single-cell RNA sequencing study uncovered a variety of subtypes with distinct phenotypes, for example cyHSC and myHSC (cytokine producing and myofibroblastic HSCs).[49]

If tissue injury and inflammation persist beyond the resolution phase, *i.e.* in 'wounds that do not heal', macrophages often switch to an immune suppressive phenotype, in the body's attempt to prevent tissue or organ disassembly. This phenotypic switch is linked to the increased production of immune suppressive cytokines, prominently TGF-β and IL-13, that suppress destructive T-cell activation and strongly promote the fibrogenic response of (myo-)fibroblasts, resulting in continuous and excessive deposition of collagen coupled with impaired degradation. This macrophage phenotype is a key player in progressive fibrosis, as long as the primary injury persists (Fig. 2).[50]

In summary, the outcome of tissue repair, whether it leads to healing and regained homeostasis or fibrosis, is primarily determined by the duration and intensity of the injury and of the resulting shift of the inflammatory response. There is a critical window of time before fibrosis develops during which coordination between resident cells and the immune response restores tissue integrity. This window can vary depending on the specific context, duration of injury and underlying cause. Timely and appropriate medical intervention, as well as individual factors (genetics, overall health, pre-existing conditions), play critical roles in determining the ultimate outcome. In general, persistent or repetitive injuries lead to chronic inflammation, whereas transient and small injuries lead to healing. Most diseases characterised by continuous or repetitive tissue damage and chronic inflammation are likely to develop into advanced fibrosis, such as alcohol-related liver disease (ALD), metabolic dysfunction-associated steatotic liver disease (MASLD), chronic viral hepatitis B or C, autoimmune and biliary liver diseases, idiopathic pulmonary fibrosis (IPF), and diabetic interstitial kidney disease among others.

Hot and cold fibrosis: Immune contexture

Despite the overall complexity of immune responses and macrophage phenotypes, the macrophage-fibroblast interplay is a key element in wound healing and fibrosis, including the growth of many solid cancers. The underlying concept has contributed significantly to a better understanding of fibrosis progression and regression from the perspective of immune status and has begun to bear fruit in the development of targeted strategies to effectively treat active fibrotic diseases. Describing fibrosis as the product of discernible and dynamically different states – *i.e.* as three outcomes of three fibrosis endotypes defined as "hot fibrosis", "cold fibrosis" and normal

wound healing with return to homeostasis – will likely guide the detection of endotype-specific therapeutic targets and drive the development of endotype-specific antifibrotic therapies.[22]

Hot fibrosis is characterised by the presence of various immune cells, including monocytes/macrophages, T cells, (myo-)fibroblasts and often also neutrophils and fibroblasts. The term "hot" is derived from the classical acute inflammatory state with the key features of redness, swelling and heat that are associated with increased blood flow, with immune cell and plasma extravasation resulting in local warming and a sensation of heat.[51] The immune response in hot fibrosis can be both beneficial and detrimental. On the one hand, immune cells help clear the damaged tissue and promote tissue repair.[46] On the other hand, chronic inflammation is responsible for 'wounds that do not heal', characteristic of advanced fibrosis and most solid cancer microenvironments.[5,20,27,28] In both cases, monocytes/macrophages are considered the most relevant immune cells involved in hot fibrosis, since their plasticity allows them to switch between different phenotypes. Therefore, much attention has recently been directed to therapeutic strategies based on macrophage switching in hot fibrosis, as it relates to fibrosis that is still densely populated by immune cells, and how to stimulate them to break down the excess ECM and serve as pro-resolution and antifibrotic macrophages (Fig. 3).[50,52–55]

In contrast, cold fibrosis is characterised by immune cell exclusion, leading to a low inflammatory environment in which the fibrotic tissue is relatively quiescent. The absence or limited presence of immune cells, combined with auto- and paracrine fibrogenic signalling, mainly among mesenchymal cells, which is amplified by their interaction with a stiff ECM,[3,56] contributes to the persistence of fibrosis and impaired tissue repair. Cold fibrosis is associated with poor prognosis and limited response to antifibrotic therapies.[17,50]

The current literature using the terms "hot" and "cold" fibrosis is scarce and mostly based on complex mathematical models describing the dynamic processes underlying the interplay between macrophages and (myo-)fibroblasts.[22,26] The models encompass macrophage and (myo-)fibroblast numbers at the injury site, proliferation, and removal of cells via endocytosis and apoptosis and the secretion of growth factors. Based on these data, the conditions in which the three different states of tissue repair, "healing", "hot fibrosis", "cold fibrosis" are stabilised can be represented in a circuit plot with macrophage and (myo-)fibroblast concentrations on the x and y axis, respectively, and a line separating the different states of the model, called a separatrix. The separatrix defines a boundary or threshold of transition between healing and fibrosis and is useful to predict the conditions that favour healing when fibrosis is likely to occur and to help define the conditions that determine whether fibrosis is associated with an active, inflamed microenvironment (hot fibrosis) or a quiescent, non-inflammatory state (cold fibrosis).[22,26]

The macrophage-fibroblast circuit models seem to be useful to describe how immune cells interact and account for different fibrosis states.[22] However, they fail to describe the structure and composition of the ECM in the different fibrotic states (hot, cold, and healing). Currently, no literature has explored how different ECM elements in the microenvironment are engaged in hot and cold fibrosis. Identifying a characteristic "hot

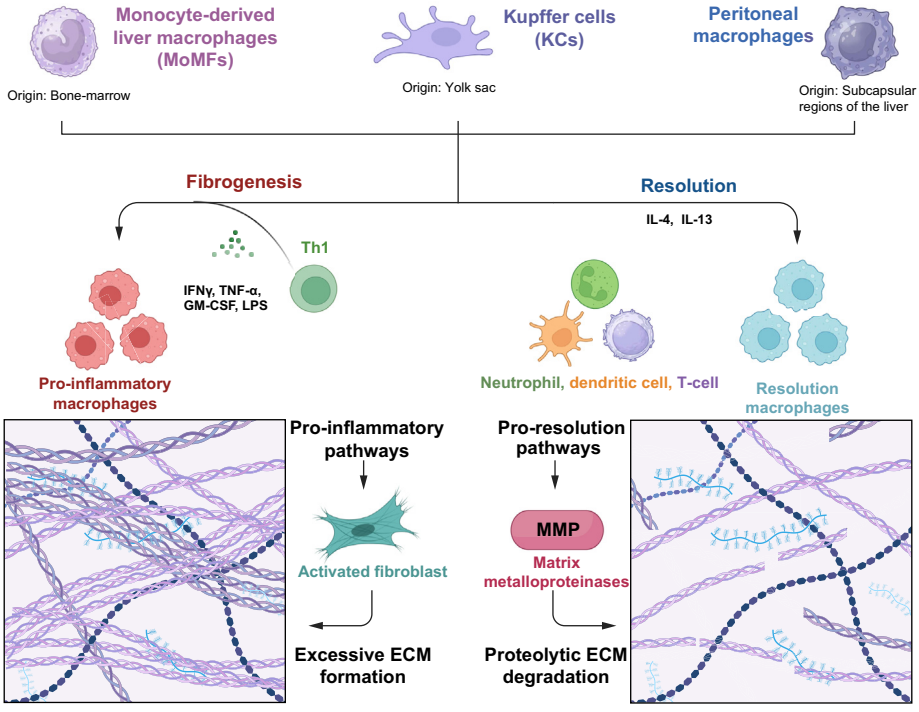

Fig. 3. Liver macrophage origins and phenotypes associated to inflammation, fibrosis development and fibrosis resolution. Therapeutic strategies to treat and prevent fibrosis through the immune system: (i) inhibiting pro-inflammatory pathways associated to fibrogenesis and ECM formation; (ii) inducing an anti-inflammatory and pro-resolution state by targeting immune cells to produce MMPs and other proteolytic enzymes to degrade the excessive fibrotic tissue and restore tissue functionality. Figure created with Biorender. ECM, extracellular matrix; MMPs, matrix metalloproteases.

fibrosis" microenvironment populated with numerous and key immune cells is highly relevant in deciding which immune cells should be targeted and what molecular targets should be switched from pro-fibrotic/inflammation inducers to a pro-resolution state, including the controlled release of proteases to degrade the excessive ECM within the fibrotic tissue (Fig. 3).[6,57] Such antifibrotic approaches would need to be complemented by strategies to repopulate cold scar tissue with pro-resolution macrophages, to reverse inactive advanced fibrosis, which is a concept that warrants further research.

Role of collagens in modulating the immune system

Collagen and especially collagen fragments with signalling properties can be crucial for the regulation of intracellular pathways connected to pathologies. For example, type VI collagen can be cleaved to release endotrophin (the carboxyterminal propeptide of the alpha 3 (VI) chain), an important fragment with endocrine functions in adipose tissue and a mediator of local fibrosis and tumour progression in obese individuals.[58–61] The signalling fragments endostatin, vastatin and tumstatin formed from type XVIII, VIII and IV collagen, respectively, have demonstrated anti-angiogenic properties. Collagens modulate immune cell behaviour via their ability to directly interact/adhere to specific receptors located on the cellular surface, such as integrins, discoidin domain receptors (DDRs) and leukocyte-associated Ig-like receptor-1 (LAIR-1), see Table 1. These interactions can influence immune cell activation, migration, and cytokine production.

Immune modulation via collagen receptors

Integrins were the first type of receptors identified to interact with collagens and are referred to as "the classical collagen receptors".[62] Integrins are essential for tissue repair and play an important role in the development of fibrosis and tumours.[63] Several integrins are expressed on activated T cells and macrophages.[64] The $\alpha1\beta1$ integrin receptor binds fibrillar collagen and controls MMP expression and synthesis of collagen.[65] Integrin a_vb_3 exhibits affinity to denatured collagen type I and specific sites in type I collagen known as RGD (arginylglycylaspartic acid),[66] thereby facilitating the interaction of cells with damaged and degraded ECM.[67]

DDRs are another type of collagen binding receptors and are members of the receptor tyrosine kinase family. They are generally divided into two main types: DDR1 and DDR2. Their stimulation induces pro-inflammatory mediators of cell proliferation and T-cell migration.[68] DDR1 is mainly expressed on epithelial cells and macrophages and is activated by most types of collagens, located in both the interstitial space and basement membranes. DDR2 is specifically expressed on (myo-)fibroblasts and other mesenchymal cells, and is exclusively activated by fibrillar collagens (type I, III and X).[69] DDRs typically do not interact with collagens unless there is a chronic pathology triggering an acute inflammatory response, for example in infections or cancer.[65] Collagen-induced DDR1 activation in macrophages promotes the production of profibrotic factors, exacerbating fibrosis. On the other hand, DDR2 stimulation induces the expression of MMPs. In liver diseases, DDR2 mediates the expression of the type IV collagenase (gelatinase) MMP-2 in activated HSCs in response to type I collagen during liver injury,[70] as well as the expression of membrane type-1 MMP (MT1-MMP, MMP-14) in fibroblasts when stimulated by a collagen-rich environment.[71]

In contrast to the pro-inflammatory response of integrins and DDRs, LAIR-1 is an inhibitory receptor which is expressed by most immune cells to prevent uncontrolled inflammation through a negative feedback loop.[72] However, its immune suppressive potential is limited, and it often indicates progressive fibrosis or cancer growth.[73,74] Hence, abnormally increased LAIR-1 expression has been linked to several chronic inflammatory diseases and has been suggested as a novel biomarker for detecting liver damage in patients with cirrhosis.[73] Fragments of collagen can also modulate inflammatory responses via Toll-like receptors,[67,75,76] which are pattern recognition receptors that recognise endogenous and exogenous danger signals associated with the presence of bacterial and viral invaders, allowing an immediate protective immune response, but also regulating wound healing and tissue repair.[75,76] Toll-like receptor 4 is expressed by activated HSCs and hepatic immune cells and is often a cofactor in the development and progression of liver fibrosis, prominently in ALD and MASLD.[67,75] Other examples of collagen binding receptors are OSCAR (osteoclast-associated receptor) and MR (mineralocorticoid receptors), which are also activated by ligands like type I and II collagens.[64,77,78]

Collagen density modulates immune cell behaviour

Mechanobiology is another hallmark in the pathogenesis of hepatic diseases. Disruption of mechanical homeostasis in the liver ECM impacts the biochemical signals produced by cells upon divergent physical forces (mechanotransduction).[79,80] Collagens act as a physical barrier, limiting immune cell infiltration into fibrotic tissues. During fibrosis, the distribution and accumulation of different types of collagens in the basement membrane and interstitial space changes their functionality as scaffolds for cells and their properties as a (partly permeable) physical barrier, impacting their ability to guide cell migration, immune cell infiltration and vascularisation.[64,80–82] The dense collagen network of fibrosis and an encapsulated cancer can impede immune cell migration and hamper the delivery of therapeutic agents into the fibrotic environment, leading to immune cell exclusion in cold fibrosis. Moreover, matrix stiffness also impacts cell behaviour via mechanical signals – mainly mediated by collagen binding to integrin receptors – that are responsible for cell proliferation, growth, viability, differentiation, apoptosis and fibrogenesis.[83–85] It is important to mention that recent advances in determining the hepatic "matrisome" could help to identify other molecules beyond collagens that are relevant in our understanding of the biomechanical properties of the liver ECM in homeostasis and disease.[79]

Collagen, fibrosis biomarkers and immunology

The activation state of immune cells can disrupt ECM homeostasis. For example, the activation state of macrophages under inflammatory conditions sets the stage for fibrosis to arise with persistent fibroblast activation, while in the pro-resolution state, macrophages can be the source of numerous matrix-degrading enzymes. As previously described, fibrosis starts in the basement membranes and then

Table 1. Collagen fragments with signalling properties and target receptors for modulation of immune cell activity.

Collagen-binding receptor	Immune cell type	Main examples	Collagen	Biological process	Disease
Integrins	Macrophages, cancer cells, leucocytes *platelets, fibroblasts, bone cells,	α1β1	Type I, IV and V I, IX, XIII and XVI	Proliferation of living cells, MMP expression and collagen remodelling	Arthritis, osteoarthritis, fibrosis (kidney), cancers, atherosclerosis[169,170]
		α2β1	Type I, II, III, V, VI, VIII and XI	Cell signals, migration, survival and differentiation; collagen remodelling	Kidney fibrosis, arthritis, colitis, insulin resistance; cancers[63,65,170]
		α10β1	Mainly type II. Other types: I, IV and V	Chondrocyte receptor; cartilage formation	Osteoarthritis[63]
		α11β1	Type I, XIII, XVII and XIX	Cell signals, migration, survival and differentiation	Fibrosis (heart), cancers[63]
Discoidin domain receptors (DDR)	Macrophages, T cells, cancer cells, leucocytes, NK cells	DDR1	Type I and IV	Cell proliferation, survival, homing, and colonisation; differentiation, migration, inflammatory response; neutrophil extracellular traps	Atherosclerosis, lung fibrosis, infections (pulmonary, kidney and vascular)[65,171]
		DDR2	Type I, II, and X	MMP expression; neutrophil migration	Arthritis, wound healing, and tumours[172]
Leukocyte receptor complex (LRC)	Leucocytes, T cells, monocytes, macrophages, activated neutrophils, NK cells, B cells, dendritic cells *osteoclasts, platelets	Leukocyte-associated Ig-like receptor-1 (LAIR-1)	Mainly type II and III. Other types: I, IV, and V	Autoimmunity, antiviral immunity, and graft tolerance	Renal diseases, rheumatoid arthritis, cirrhosis[73]
		Osteoclast-associated receptor (OSCAR)	Type I and II	Osteoimmunology, osteoclastogenesis	Rheumatoid arthritis[77,78]
		Glycoprotein VI (GPVI)	Type I	Platelet activation; platelet aggregate formation	Cardiovascular and thrombotic diseases[173]
Toll-like receptors (TLR)	Macrophages, dendritic cells *fibroblasts, epithelial cells, hepatic stellate cells, hepatocytes		Type I, II, VI	Inflammation, innate immune response, activation of hepatic stellate cells	Liver fibrosis, arthritis, obesity, adipose tissue fibrosis, gut inflammation, renal fibrogenesis[173]

*Other cell types that are not immune cells.

propagates to the interstitial space. Two different compartments of the ECM are impacted by different biological processes: (i) degradation and remodelling of type IV collagen in the basement membrane via MMPs to facilitate the recruitment of immune cells and (ii) excessive synthesis/formation of fibrillar collagens in the interstitial space from activated fibroblasts. In both scenarios, unique collagen fragments are produced in the ECM environment surrounding the inflamed/injured site and reach the bloodstream, where they can be non-invasively measured to assess changes in ECM composition as well as in the activation state of the lesion's immune cells, see Fig. 4.[9,86–88] It is important to highlight that besides monitoring specific non-invasive collagen-based biomarkers associated with pathologies, significant advances have been made using proteomics-based techniques to unravel ECM composition through the "matrisome", which is a promising tool to enhance the identification of new biomarkers and treatment targets.[89]

Therefore, biomarkers reflecting ECM remodelling have significant potential to assess disease activity at a systemic level, as well as at the level of the microenvironment affected by injury, fibrosis and inflammation. Collagen-based biomarkers seem to be more versatile than most current inflammatory markers in the clinic as they have demonstrated a broader diagnostic utility. Thus, they i) provide insights into the interconnected processes of fibrosis, inflammation, and resolution; ii) have great potential for assessing disease prognosis; iii) can help to thoroughly evaluate treatment response; iv) can distinguish disease phenotypes; and v) facilitate the selection and monitoring of the most effective treatment for each patient (Table 2).

In general, the context of use and understanding of the biology behind biomarkers are extremely important elements for selecting the most suitable biomarkers to answer specific research questions within fibro-inflammatory diseases.[90] For example, although the formation of both type I and type III collagen is significantly enhanced in fibrosis, biomarkers reflecting type III collagen synthesis were proven to have superior specificity for assessment of hepatic and pulmonary fibrosis. The lack of specificity of type I collagen formation markers in such pathologies is due to type I collagen being the most abundant type, being produced throughout all body tissues, including bone. On the other hand, enhanced type III collagen formation is an exclusive feature of soft tissues, so that its serum markers reflect fibrosis dynamics in larger organs, and prominently in liver fibrosis.[91–95] Endotrophin is also a good biomarker in liver diseases,[60,96,97] as well as being a key novel biomarker for predicting morbidity and mortality in cardiovascular, renal and general autoimmune diseases.[59] These examples underscore the need for new studies to further explore the utility of the many different collagen biomarkers in the context of hot and cold fibrosis in different organs.

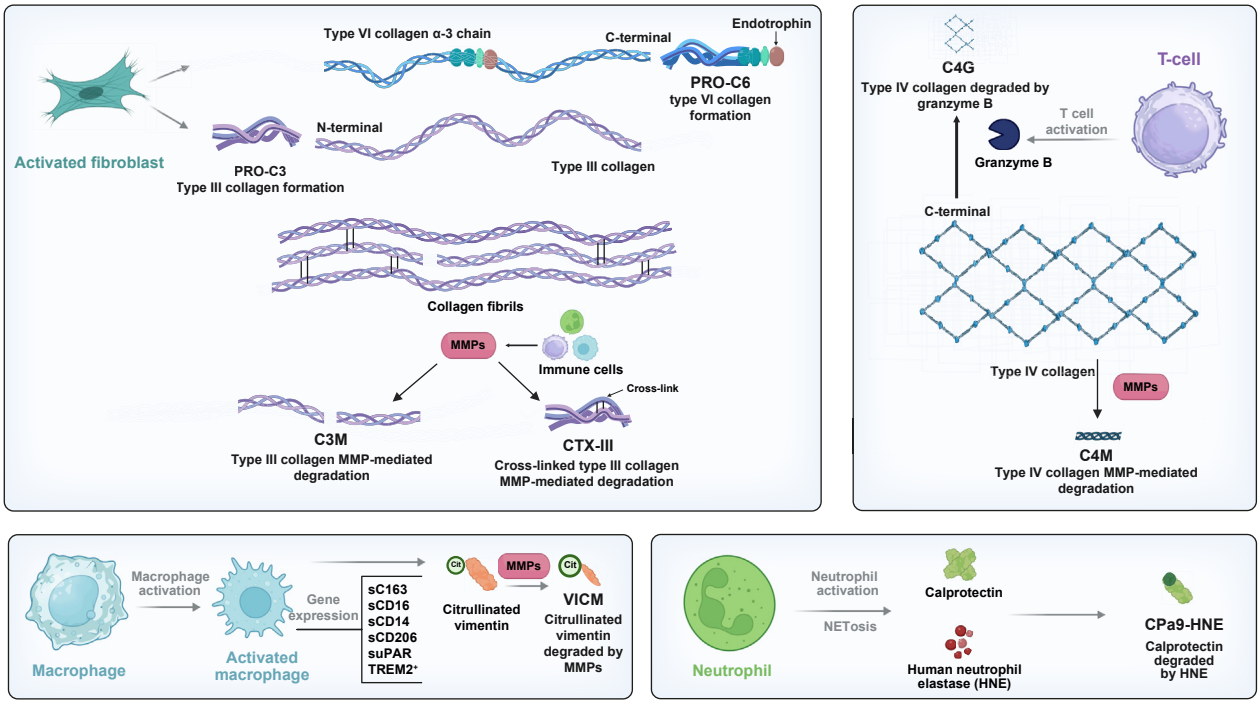

Fig. 4. Cells as a source of non-invasive biomarkers for future investigation of hot & cold fibrosis. Based on fragments of ECM proteins and peptides that reflect fibrogenesis/fibroblast activation (type III and VI collagen formation), fibrolysis (degradation of type III and type IV collagen by matrix metalloproteinases) and immune cell activity (neutrophils, T-cells and macrophages) as well as based on subpopulations of macrophages determined by gene expression that are relevant for hepatic diseases (sCD163, sCD16, sCD14, sCD206, suPAR, TREM2^{+}). C3M, fragment of degradation of type III collagen via MMPs; C4M, fragment of degradation of type IV collagen via MMPs; C4G, fragment of degradation of type IV collagen by Granzyme B; CTX-III, fragment of type III collagen cross-linked via lysil oxidase (LOX) and degraded via MMPs; CPa9-HNE, fragment formed from degradation of calprotectin via neutrophil elastase; ECM, extracellular matrix; MMPs, matrix metalloproteinases; PRO-C3, fragment of N-terminal type III collagen; PRO-C6, fragment of C-terminal alpha 3 chain of type VI collagen; VICM, fragment of degradation of citrullinated vimentin by MMPs. Figure created with Biorender.

Table 2. Overview of ECM and macrophage biomarkers relation to immune cells and their biological role within different fibrotic and inflammatory diseases.

ECM component	Biomarker	Production	Biological process	Cell type	Tissue/disease	Outcomes of interest	Literature
Type I collagen	C1M	Degradation via MMP	Tissue degradation, inflammation	Macrophages	Joint tissue - synovial inflammation/rheumatoid arthritis Lung – idiopathic pulmonary fibrosis, cancer Skin – hidradenitis suppurativa Heart – atherosclerosis, myocardial infarction	Disease activity, local tissue degradation, identification of disease progressors, treatment response, prognostic value (outcomes, death)	Siebuhr *et al.*, 2013.[127,174] Thudium *et al.*, 2020.[175] Willumsen *et al.*, 2014.[86] Organ *et al.*, 2019.[86] Villumsen *et al.*, 2014.[86] Groen *et al.*, 2022.[176] Holm Nielsen *et al.*, 2018[177] Bertelsen *et al.*, 2018.[178]
Type II collagen	C2M	Degradation via MMP	Tissue degradation, inflammation	Macrophages	Cartilage degradation - rheumatoid arthritis	Disease activity, identification of disease progressors, treatment response	Bay-Jensen *et al.*, 2013.[129] Maijer *et al.*, 2016.[179] Qvist *et al.*, 2009.[180] Gudmann *et al.*, 2014.[181]
Type III collagen	PRO-C3	Type III collagen synthesis	Fibrogenesis	Fibroblasts	Liver – fibrosis, MASLD, MASH, ALD, cirrhosis, HCC Lung – COPD, IPF	Disease activity, identification of disease progressors, treatment response, prognostic value (outcomes, death)	Nielsen *et al.*, 2013.[182] Daniels *et al.*, 2019.[95] Thiele *et al.*, 2021.[95] Nielsen *et al.*, 2014.[110] Karsdal *et al.*, 2020.[9] Karsdal *et al.*, 2016.[183] Boyle *et al.*, 2019.[94] Stoltz *et al.*, 2017.[184] Sand *et al.*, 2015.[185]
	C3M	Degradation via MMP	Tissue degradation, inflammation	Macrophages	Liver – MASLD, cirrhosis, ALD, HBV and HCV Joint tissue - synovial inflammation/rheumatoid arthritis Breast cancer	Disease activity, identification of disease progressors, treatment response	Prattiknjo *et al.*, 2018.[186] Thiele *et al.*, 2021.[107] Nielsen *et al.*, 2016.[126] Maijer *et al.*, 2016.[179] Banys-Paluchowski *et al.*, 2019.[187]
	CTX-III	Cross-linking via lysil oxidase (LOX) Degradation via MMP	Fibrosis resolution	Unknown	Liver – MASLD, chronic liver disease	Identification of disease progressors, intervention response	Pehrsson *et al.*, 2022.[8]
Type IV collagen	C4M	Degradation via MMP	Tissue degradation, inflammation	Macrophages	Liver – fibrosis, cirrhosis Joint tissue - synovial inflammation/rheumatoid arthritis Breast cancer	Disease activity, early diagnostic, treatment response	Løsmann *et al.*, 2023.[162] Lehmann *et al.*, 2019.[188] Banys-Paluchowski *et al.*, 2019.[187]
	C4G	Degradation via granzyme-B degradation	T-cell infiltration	T cells	Cancer	Identification of patients responding to therapy	Jensen *et al.*, 2020.[158] Hasselbalch et at 2023.[189] Christensen *et al.*, 2022.[190]
Type VI collagen	PRO-C6	Type VI collagen synthesis	Fibrogenesis	Fibroblasts	Liver – fibrosis, MASLD, MASH, ALD, cirrhosis Heart – CVD Kidney – CKD Pancreas – cancer	Identification of disease progressors, treatment response, prognostic value (outcomes, death, disease recurrence)	Luo *et al.*, 2018.[91] Kerbert *et al.*, 2021.[96] Engel *et al.*, 2023.[191] Johansen *et al.*, 2023.[93] Rasmussen *et al.*, 2018.[192] Chirinos *et al.*, 2022.[193] Wiese *et al.*, 2020.[194] Pedersen *et al.*, 2022.[195]
	C6M		Tissue degradation	Macrophages	Lung – COPD, IPF	Disease activity, identification of disease progressors, treatment response	Jenkins *et al.*, 2015.[137] Sand *et al.*, 2015.[185] Bihlet *et al.*, 2016.[196]
	Endotrophin		Fibroinflammatory marker, signalling	Adipocytes	Liver – MASLD, cirrhosis, hepatitis B and C, HCC Adipose tissue – metabolic disfunction, insulin resistance, T2D Lung – COPD Heart - HFpEF	Outcome prediction (death, survival, complications)	Leeming *et al.*, 2020.[97] Henriksen *et al.*, 2023[61] Kim *et al.*, 2020.[120] Sun *et al.*, 2014.[60] Karsdal *et al.*, 2017.[197] Rønnow *et al.*, 2020.[198]

(*continued on next page*)

Table 2. (*continued*)

ECM component	Biomarker	Production	Biological process	Cell type	Tissue/disease	Outcomes of interest	Literature
Type VIII collagen	Vastatin	Type VIII collagen degradation	Anti-angiogenic signalling	Unknown	Endothelial tissue Liver – HCC Intestine – colorectal cancer, UC	Anti-angiogenesis, treatment, patient stratification	Lindholm *et al.*, 2019.[199] Shen *et al.*, 2016.[200] Villumsen *et al.*, 2018.[201]
Type XVIII collagen	Endostatin	Degradation via MMP and cathepsin	Anti-angiogenic signalling	Endothelial cells, fibrochondrocytes and chondrocytes	Cancer – angiogenesis inhibitors of cancer Retinal epithelium – age dependent vision loss	Cancer therapy, visual function	Folkman 2006.[202] Maneros & Olsen, 2005.[203]
Calprotectin	CPa9-HNE	Calprotectin formation, degradation neutrophil elastase	Immune response, inflammation	Neutrophils	Intestine – IBD, UC, Crohn's disease Lung – COPD, IPF Cancer	Disease activity and severity, patient stratification, treatment response, outcome prediction	Bourgonje *et al.*, 2022.[204] Mortensen *et al.*, 2021.[205] Mortensen *et al.*, 2022.[151] Alexdottir *et al.*, 2022.[206] Hansen *et al.*, 2023.[207] Jensen *et al.*, 2021.[208]
Macrophages	VICM	Citrullination of vimentin, degradation via MMP	Macrophage activation	Macrophages	Liver – MASLD, HCV, PBC Lung – cancer, COPD, IPF Intestine – IBD, Chron's disease, UC	Disease activity and progression, patient stratification, treatment response	Vassiliadis *et al.*, 2012.[138] Vesterhus *et al.*, 2021.[92] Willumsen *et al.*,2014.[86] Nissen *et al.*, 2019.[209] Simões *et al.*, 2023.[210] Mortensen *et al.*, 2015.[211]
	sCD16	Soluble Fc gamma Receptor III (sFcγRIII)	Macrophage activation, inflammation	Macrophages, monocytes and natural killer cells	Liver – MASLD, MASH	Liver fibrosis	Lambrecht & Tacke, 2021[212]
	sCD14	Co-receptor for recognising bacterial lipopolysaccharides (LPS)	Immune cell activation	Macrophages and monocytes	Liver – MASLD, MASH, ALD, HCV, HBV	Metabolic, inflammation,MASLD, disease severity, gut-liver axis and sepsis, fibrosis severity	Lambrecht & Tacke, 2021[212]
	sCD163	High-affinity scavenger receptor for the hemoglobin-haptoglobin complex	Kupffer cell activation	Kupffer cells, monocytes	Liver – MASLD, MASH, ALD, HCC, acute liver failure	Fibrosis stage, disease severity and progression, treatment response, outcome prediction	Lambrecht & Tacke, 2021[212] Nielsen *et al.*, 2021[213]
	sCD206	Soluble form of dipeptidyl peptidase-4 (DPP4)	Macrophage activation, HSC activation	Macrophages	Liver – MASLD, MASH, fibrosis, HCC	Metabolic dysfunction, insulin resistance, tumour proliferation, MASH progression	Nielsen *et al.*, 2021[213]
	suPAR	Soluble urokinase plasminogen activator receptor	Macrophage activation, HSC activation, tissue remodelling	Macrophages	Liver – MASLD, MASH, ALD, HCC, HCV, HBV	Impaired liver function, risk of mortality, outcome prediction	Zimmermann *et al.*, 2013[146]
	sTREM2^{+}	Expression of soluble triggering receptor expressed on myeloid cells 2	Macrophage activation, regulation of inflammation, lipid metabolism	Macrophages	Liver – MASLD, cirrhosis, MASH	Disease severity, outcome prediction, local fibrosis	Ganguly *et al.*, 2024[32] Hendrikx *et al.*, 2022[147]

ALD, alcohol-related liver disease; CKD, chronic kidney disease; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; IPF, idiophatic pulmonary fibrosis; HCC, hepatocellular carcinoma; HFpEF, heart failure with preserved ejection fraction; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, Metabolic dysfunction-associated steatohepatitis; PBC, primary biliary cholangitis; T2D, type 2 diabetes; UC, ulcerative colitis.

In the subsequent sections, we discuss a spectrum of collagen biomarkers indicative of inflammatory processes associated with fibrosis, focusing on their specificity for several pathologies (see the distribution of biomarkers in different disease areas in Fig. 5 and a detailed description of biomarkers found in the literature in the supplementary material, Table S1). We will place an increased emphasis on liver fibrosis, given that the inflammatory mechanisms underlying it remain complex and incompletely understood yet possess significant potential for future therapeutic strategies.

Biomarkers of ECM formation in fibrogenesis and inflammation: A focus in chronic liver diseases and utility in other chronic pathologies

Liver fibrosis results from repeated cell injury due to excessive alcohol consumption, metabolic disorders, viral hepatitis, autoimmune diseases and exposure to toxins and drugs.[98] Among these different aetiologies, MASLD has been recognised as the leading cause of all liver-related morbidity and mortality. MASLD progression comprises a broad spectrum which includes the accumulation of lipid droplets in the hepatocytes in steatosis, chronic inflammation characterised by hepatocyte ballooning and lobular inflammation, development of liver fibrosis as a consequence of sustained inflammation and tissue injury, progression to cirrhosis and further development of hepatocellular carcinoma (HCC).[98–100] The onset of MASLD is characterised by an initial inflammatory state in response to the liver injury when inflammatory cells initiate tissue repair processes, which are subsequently disrupted by increased ECM remodelling and tissue turnover, resulting in excessive deposition of type I and type III collagen and ultimately fibrosis, see Fig. 1. In this context, collagen epitope biomarkers have been proven to be effective diagnostic and prognostic tools for MASLD, as well as for monitoring the efficacy of therapeutic interventions.[9,21,100–102]

The N-terminal pro-peptide of type III collagen biomarker (PRO-C3) has been widely described as a systemic biomarker for measuring the formation of type III collagen and HSC activation in different aetiologies of liver fibrosis: MASLD,[94,95,103–105] MASH,[91,105,106] ALD,[93,107] cholestatic[108] and viral liver diseases.[109,110] Serum and plasma levels of PRO-C3 have been shown to correlate with the severity of fibrosis and have demonstrated superior diagnostic performance to identify patients with advanced fibrosis, which is classified as fibrosis stage 3 and 4 (F3 and F4, respectively), according to the histological scoring system established by Kleiner.[94,95,105,111] Blood levels of PRO-C3 were also found to correlate with MASLD disease activity and to have prognostic utility for stratifying patients according to the risk of developing clinical outcomes (liver-related or not) in full spectrum fibrosis.[112] Correlations between PRO-C3 levels and systemic inflammation measured through C-reactive protein (CRP) have also been observed in patients with cirrhosis.[113] Although the levels of PRO-C3 are consistently high in patients with advanced liver fibrosis, the baseline levels of PRO-C3 vary across different aetiologies, suggesting that the levels of PRO-C3 reflect differences in pro-inflammatory environments and fibroblast activity due to specific underlying causes of disease and patient characteristics.[112]

Determining levels and dynamic ranges of PRO-C3 according to different types of liver injury could be a promising tool for monitoring and selecting patients for clinical trials. Importantly, PRO-C3 has been shown to be an efficient serological marker to predict response to antifibrotic therapy. In this context, patients with progressive liver fibrosis who exhibit PRO-C3 levels above a certain cut-off indicating hot fibrosis are more likely to respond to antifibrotic therapy, for example farglitazar[114] and pegbelfermin,[115,116] with a reduction of both PRO-C3 and histological stage on follow-up liver biopsy.[117] Baseline PRO-C3 levels have also been shown to be a good tool for identifying patients who better respond to resmetirom in a phase II trial of patients with MASH.[117] Moreover, serum PRO-C3 allows for the assessment of the decrease in fibrogenic activity in patients with cirrhosis upon autologous

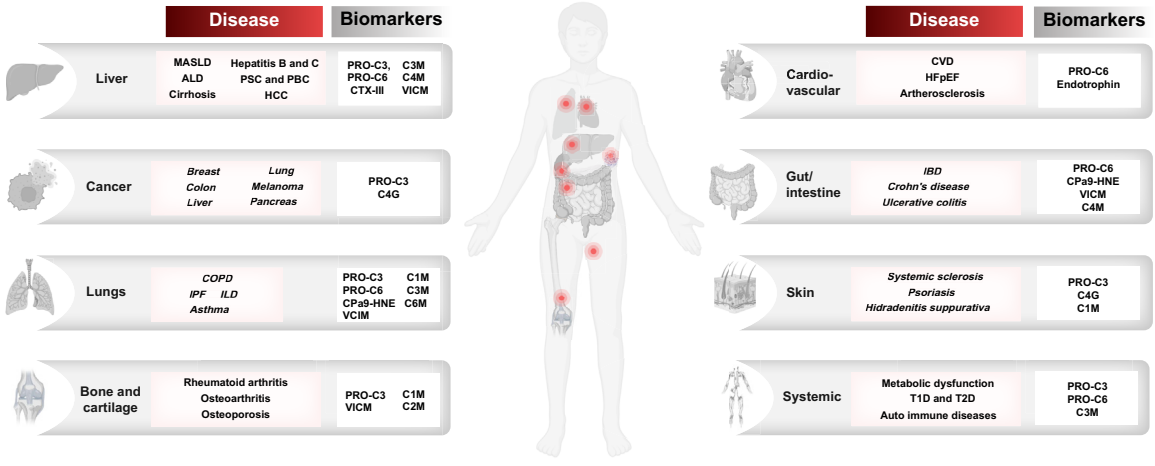

Fig. 5. Distribution of biomarkers based on ECM fragments across different disease areas. ALD, alcohol-related liver disease; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; ECM, extracellular matrix; IBD, inflammatory bowel disease; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; HCC, hepatocellular carcinoma; HFpEF, heart failure with preserved ejection fraction; HS, hidradenitis suppurativa; MASLD, metabolic dysfunction–associated steatotic liver disease; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; T1D, type 1 diabetes; T2D, type 2 diabetes; RA, rheumatoid arthritis; UC, ulcerative colitis. Figure created with Biorender.

macrophage therapy, a treatment where infused macrophages released MMPs to degrade (cold) fibrotic liver tissue.[118]

In patients with MASLD, high levels of endotrophin are highly correlated with hepatic inflammation, hepatic fat accumulation, impaired insulin sensitivity, angiogenesis, and adipose tissue inflammation.[60,119–121] Elevated serum levels of PRO-C6 (fragment of C-terminal alpha 3 chain of type VI collagen), a biomarker reflecting the formation of type VI collagen and endotrophin, were found to correlate with inflammatory biomarkers represented by cytokines IL-8 and IL-10, and M2-type macrophage activation marker CD163, and to be highly expressed in specific areas from liver biopsies where activated HSCs are present.[96,113] Due to the relevance of endotrophin for metabolic disorders, PRO-C6 is an exceptional prognostic biomarker for patients with liver diseases and allows patients with liver disease to be stratified by their risk of metabolic-related outcomes like cardiovascular events and MASH (previously named non-alcoholic steatohepatitis - NASH).[121] Kerbert *et al.*, reported that the severity of chronic systemic inflammation correlates with levels of PRO-C6 and reflects the risk of outcomes, with PRO-C6 displaying superior prognostic utility to stratify patients at risk of developing hepatic and extrahepatic organ failure and patients with acute-on-chronic-liver-failure at risk of mortality.[96] Moreover, high levels of endotrophin were also found to correlate with the presence of HCC and tumour growth in patients with HCC and animal models of liver cancer.[97] Endotrophin has been recognised as a unique driver of fibro-inflammatory diseases and must be included in studies aiming to explore the concept of hot and cold fibrosis in liver diseases.

Biomarkers of ECM degradation for inflammation, immune cell activation and fibrosis resolution

Some of the well-established biomarkers for assessing acute inflammation in the general population lack specificity to identify the origin and cause of the inflammatory process. This is the case for CRP, which is widely used in clinical practice to monitor acute systemic inflammation. In chronic inflammatory diseases, activated immune cells secrete enzymes that degrade ECM in the affected tissues, yielding different types of biomarkers that may indicate specific pathways for proteolytic cleavage of collagens or proteins and correlate with both systemic and local inflammation in specific tissue environments.[122,123] Herein, we discuss the utility of biomarkers formed by these specific degradation pathways to assess inflammatory activity and potential correlations to fibrosis development, as well as to provide prognostic value and tools for phenotyping different disease profiles or monitoring treatment response.

MMP-mediated degradation of collagens: the fine line between inflammation and fibrosis

Impaired ECM degradation is another hallmark behind the disruption of ECM homeostasis within chronic liver inflammation.[124] Several studies have shown that the levels of MMP-degraded collagens I, III, IV, V, VI are also significantly different across different liver disease aetiologies including ALD,[107] biliary diseases,[92] and MASLD,[125] and are usually also associated with inflammation. A biomarker reflecting the degradation of type III collagen was found to correlate with the activation of HSCs in patients with advanced chronic liver disease, thus representing a more specific ECM-derived biomarker that is released in inflammation and that correlates with CRP, a marker of systemic inflammation.[113] Such a correlation can be explained by the fact the pro-inflammatory environment in injured hepatocytes locally releases cytokines and other pro-inflammatory molecules which can both activate HSCs towards fibrogenesis and reach the blood stream triggering systemic inflammation.[113] The correlation of type III collagen degradation markers with both systemic and local inflammation can add some insights into defining fibrosis as hot or cold and help with characterising the fibrotic environment according to its inflammatory status. Biomarkers reflecting degradation of type VI collagen were also found to correlate with inflammation in patients with chronic hepatitis C, while biomarker levels reflecting the degradation of the basement membrane via type IV collagen fragments were found to correlate with disease activity and fibrosis stage in both chronic hepatitis B and C.[126]

Osteoarthritis (OA) and rheumatoid arthritis (RA) are immune-driven degenerative diseases characterised by a persistent inflammatory state featuring the upregulation of MMPs which destroy articular cartilage (primarily composed of type II collagen) and non-cartilage connective tissue (largely composed of type I collagen – the most abundant collagen in the body – as well as type III collagen). Biomarkers representing the degradation of type I, II and III collagens were found to help identify local inflammation as they reflect progressive breakdown of joint cartilage and changes in the underlying bone in OA and RA. Some OA phenotypes with persistent synovial inflammation exhibit fibrosis in the synovial membrane (soft tissue) which can be assessed by elevated levels of PRO-C3.[127,128] In RA, macrophages with pro-inflammatory, destructive and remodelling activity are the major contributors to disease progression, and the levels of degraded fragments of type II and III collagens were found to correlate with disease inflammatory activity and to help identify those with progressive disease.[129] Another important aspect of RA is that a considerable number of patients develop fibrotic conditions such as interstitial pulmonary fibrosis and myocardial fibrosis,[130–132] which confer a poor prognosis and account for the highest mortality rates in patients with RA.[133]

The degradation of collagens was also found to provide good prognostic biomarkers for lung diseases, such as chronic obstructive pulmonary disease (COPD) and IPF. COPD occurs due to a chronic destructive inflammatory response in the airways characterised by increased MMP activity with less focal fibrosis and enhanced turnover of collagens. A biomarker of degradation of type VI collagen was found to be elevated in patients with COPD and to provide a good prognostic tool to identify patients at risk of death when used individually; the combination of this biomarker with the fibrogenesis biomarker PRO-C6 and the systemic inflammation biomarker CRP was also shown to have superior prognostic performance compared to biomarkers used individually.[134] MMP-generated collagen fragments were also found to provide good prognostic tools in IPF, which is characterised by persistent microinjuries in the alveolar epithelium leading to fibrosis/scarring.[135] Biomarkers reflecting turnover of type I and type III collagen (here PRO-C3 and MMP-degraded type III collagen neoepitope marker) were linked to disease progression and

demonstrated good utility for identifying stable patients at higher risk of progressive IPF, and for monitoring responses to antifibrotic drugs like nintedanib and pirfenidone.[136] In an observational cohort study (PROFILE), biomarkers associated with MMP-mediated degradation of type I, III and VI collagens, combined with CRP (CRPM) were shown to be a novel prognostic tool to identify patients with progressive IPF and to predict survival.[137]

Biomarkers to directly monitor immune cell activity

MMPs target other proteins besides collagens, for example the citrullinated form of vimentin, which is a cytoskeletal protein mainly expressed in fibroblasts and endothelial cells. Citrullination is a post-translational modification mediated by classically activated macrophages which modifies protein-bound arginine to citrulline making the protein more prone to MMP degradation, yielding fragments derived from MMP degradation which reflect macrophage activation.[138] MMP-degraded fragments of citrullinated vimentin have been demonstrated to be associated with early fibrosis in patients with chronic hepatitis C virus infection and MASLD,[138] and have been widely described as a biomarker of macrophage activation in several inflammatory diseases[88,139–142] and as a predictor of treatment response.[140] Such fragments represent a potential biomarker to identify hot and cold fibrosis endotypes and to guide patient selection, identification of responders, and treatment monitoring, as well as to improve our understanding of the role of vimentin in HSC activation.[143] Other markers based on gene expression in KCs have been tested as biomarkers of macrophage activation and have potential prognostic value in chronic liver disease: sCD16 (inflammation and liver fibrosis);[144] sCD14 (inflammation, steatosis and liver fibrosis in MASLD);[31,144] sCD163 (resolution of inflammation and advanced disease states in MASH, viral and autoimmune disease);[144,145] sCD206 (tissue remodelling; chronic inflammation and fibrosis in MASH, cirrhosis and HCC);[144,145] soluble urokinase plasminogen activator receptor (liver fibrosis, impaired liver function, risk of mortality);[146] sTREM2+ (local fibrosis, disease severity in MASLD, cirrhosis, MASH).[147] Chronic intestinal inflammation in inflammatory bowel disease (IBD) is a major factor for the development of intestinal fibrosis.[148] Several studies designed to investigate IBD mechanisms have shown the involvement of immune cells and inflammatory responses in intestinal fibrogenesis. In fact, there is a vast literature presenting current and promising treatments for IBD-associated fibrosis by targeting mucosal immune cells such as macrophages, T cells, or innate lymphoid cells.[148,149] Recently, neutrophils have been recognised as potential targets for future IBD treatment. Neutrophils are essential to protect the gut from invading pathogens, for example through the release of NETs (neutrophil extracellular traps). In inflamed tissues, activated neutrophils secrete calprotectin, which is a biomarker of intestinal inflammation detected in blood and stool and a predictor of relapse in patients with IBD (Crohn's and ulcerative colitis).[150] Neutrophils also secrete the protease human neutrophil elastase (HNE). A neo-epitope biomarker measuring HNE-mediated degradation of calprotectin in serum has shown promising results in assessing IBD activity, identifying patients with active ulcerative colitis and Crohn's disease,[151] predicting distinct disease states in Crohn's disease (AUC of 0.98) and ulcerative colitis (AUC of 0.96), and better correlating with respective endoscopic scores than faecal calprotectin. The biomarker of HNE-degraded calprotectin has the advantage of being detectable and quantifiable in the circulation, thereby also reflecting the systemic features of IBD.

Strategies to monitor ECM degradation during fibrosis regression

Besides the fibrogenesis biomarker PRO-C3 and type III collagen-associated biomarkers that assess degradation, a fragment of the degradation of cross-linked type III collagen (CTX-III) has been investigated as a novel biomarker reflecting fibrosis resolution in patients with liver fibrosis. CTX-III levels were found to increase significantly, alongside an improvement of liver function and regression of inflammation, following bariatric surgery in patients with MASLD and severe obesity.[8] Here, the expected fibrosis regression after bariatric surgery confirms the predictive value of a regression marker like CTX-III. Finding therapies that promote the degradation of cross-linked collagens by promoting clearance of excessive ECM by immune cells is especially relevant for liver fibrosis management as the formation of collagen cross-linking is associated with increased liver stiffness/scarring.[152]

ECM turnover of solid tumours in immuno-oncology

In the field of immuno-oncology, significant progress has been made in understanding the immune landscape of solid tumours and developing strategies to overcome immune evasion. These lessons can potentially be translated to fibrosis treatment. One key concept is the immunosuppressive tumour microenvironment (TME), which shares similarities with the immune contexture in fibrosis. In both settings, immune cell exclusion, immune checkpoint activation, and the presence of immunosuppressive cells contribute to immune quiescence and treatment resistance.

Solid tumours with increased collagen density and high fibrotic activity are more resistant to immunotherapy.[153] This is because increased ECM stiffness can harbour large amounts of profibrogenic and tumour suppressive (M2-type) macrophages that suppress CD8+ cytotoxic T cell activities and mechanically prevent further tumour-infiltrating T cells from reaching the cancer tissue. The lack of T cells in the TME describes the cancer-related "cold" tumour or the immune-desert phenotype. In contrast, a phenotype characterised by the presence of M1-like immune cells with a T-cell permissive TME is often referred to as a hot tumour and is more likely to respond to immune therapy.[23]

Like solid cancers, cirrhosis is described by abnormal liver ECM stiffness with irreversible damage to tissue structure. Currently, cirrhosis cannot be cured; however, appropriate treatment can slow down disease progression and improve the quality of life of patients.[64,74,81] Without treatment, a cirrhotic liver fosters an altered microenvironment with pathological features, including fibrogenesis and immune deregulation, that contribute to a precancerous environment and further development of HCC.[154,155] In this sense, HCC and cirrhosis share common signalling pathways involving profibrogenic M2-type macrophages, mesenchymal cells,

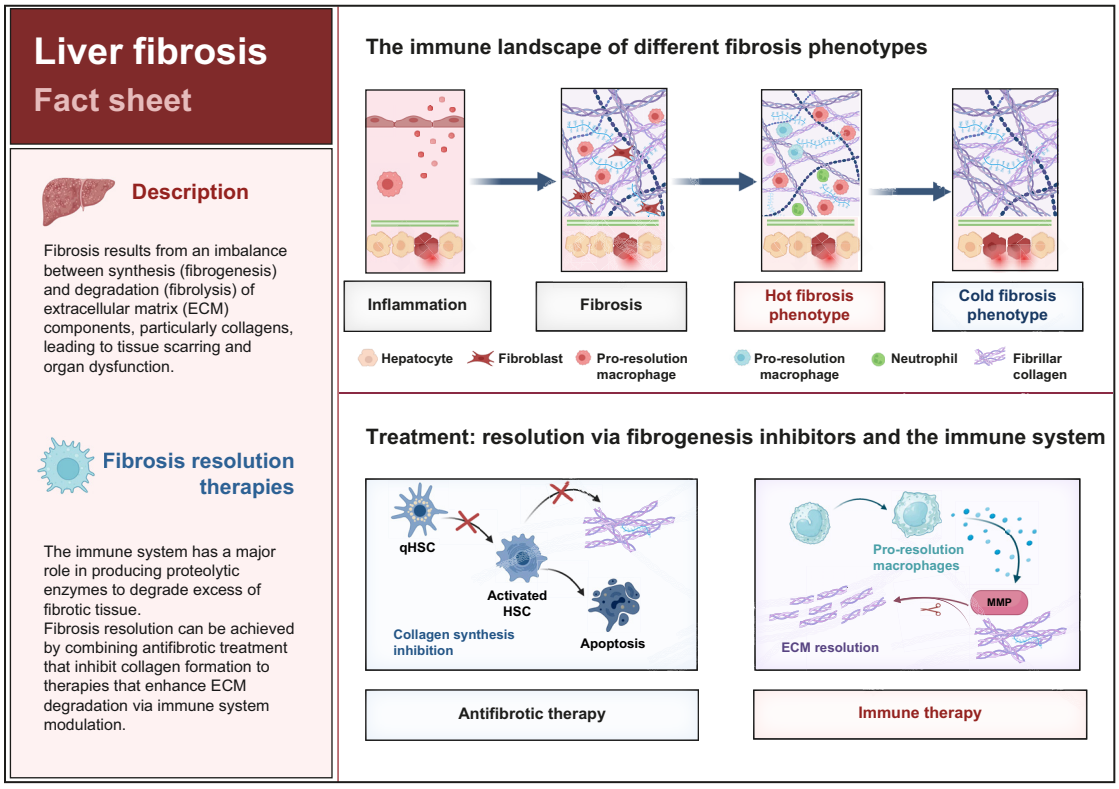
Liver fibrosis
Fact sheet
Description
Fibrosis results from an imbalance between synthesis (fibrogenesis) and degradation (fibrolysis) of extracellular matrix (ECM) components, particularly collagens, leading to tissue scarring and organ dysfunction.
Fibrosis resolution therapies
The immune system has a major role in producing proteolytic enzymes to degrade excess of fibrotic tissue.
Fibrosis resolution can be achieved by combining antifibrotic treatment that inhibit collagen formation to therapies that enhance ECM degradation via immune system modulation.
The immune landscape of different fibrosis phenotypes
Inflammation
Fibrosis
Hot fibrosis phenotype
Cold fibrosis phenotype
Hepatocyte
Fibroblast
Pro-resolution macrophage
Pro-resolution macrophage
Neutrophil
Fibrillar collagen
Treatment: resolution via fibrogenesis inhibitors and the immune system
qHSC
Activated HSC
Collagen synthesis inhibition
Apoptosis
Pro-resolution macrophages
MMP
ECM resolution
Antifibrotic therapy
Immune therapy

Fig. 6. Liver fibrosis fact sheet. Describing fibrosis through different phenotypes based on the presence of active immune cells within the fibrotic tissue and strategies for fibrosis treatment via inhibition of fibrogenesis and/or stimulation of immune system for fibrosis resolution.

cancer immune-suppressive TME and collagen binding to cell receptors.[155–157] HCC is the major cause of death in patients with compensated cirrhosis,[154,155] therefore, understanding biological mechanisms that link fibrosis to carcinogenesis is crucial to develop targeted therapies to prevent death among patients with HCC.

Strategies targeting immune checkpoints, such as PD-1/PD-L1 and CTLA-4 inhibitors, known as immune checkpoint inhibitors (ICIs), have shown efficacy in some solid tumours and have potential to treat fibrosis. A biomarker specifically reflecting type IV collagen degradation via the protein granzyme-B in combination with PRO-C3 has demonstrated potential to identify patients with melanoma responding to ICI treatment.[158] The release of granzyme-B with consequent degradation of type IV collagen in the basement membrane is a crucial process for T-cell infiltration into the TME and therapies that induce the release of granzyme-B can improve the efficacy of immune therapy. On the other hand, elevated levels of type IV collagen fragments formed via MMP degradation reflect poor prognosis (metastasis development) in patients with cancer.[159,160] In that sense, characterising the tumour microenvironment is essential for treatment decision-making.[161] In liver fibrosis, the degradation of the basement membrane has been associated with early fibrosis.[162,163] Basement membrane biomarkers measured via long non-coding RNA also have prognostic value for HCC.[164] Such differences between biomarkers derived from the same collagen type highlight the importance of developing biomarkers targeting specific biological processes. Understanding the heterogeneity within fibrotic tissues, similar to the concept of tumour heterogeneity, can guide personalised treatment approaches.

Another lesson is the importance of combining therapies to enhance the immune response. In solid tumours, combining ICIs with other treatment modalities, such as chemotherapy or radiation, has shown synergistic effects with improved efficacy in comparison to the individual therapies under certain conditions.[165–167] Similar approaches could be explored in fibrosis using therapies to modulate the immune system to overcome immune exclusion and stimulate ECM degradation, thereby enhancing the therapeutic response of antifibrotic drugs that intercept fibrogenesis.

Conclusion and future perspectives

There is a close and complex interplay between inflammation and fibrosis. A better understanding of this interplay can aid in the diagnosis and management of early inflammation to prevent the development of fibrosis and associated complications.

Hot and cold fibrosis represent distinct immune contextures within fibrotic tissues, with implications for disease progression and treatment response. Lessons learned from immuno-oncology research in solid tumours provide valuable insights into potential strategies for designing novel therapeutic strategies targeting fibro-inflammatory pathways via the immune system, including immune checkpoints, and personalised approaches based on different fibrosis endotypes, see Fig. 6.

Most of the available antifibrotic drugs focus on inhibiting myofibroblasts to decrease ECM production. However,

currently there are no effective therapies to promote fibrosis resolution, so-called pro-resolution interventions, via immune cell activation. The use of autologous macrophage therapy is one promising therapeutic approach to harness pro-resolution macrophages to degrade ECM and improve liver function in patients with cirrhosis, as demonstrated in the MATCH study.[168] A characteristic hot fibrosis phenotype comprises the immune landscape that is necessary for stimulating antifibrotic and pro-resolution macrophages. In this sense, monitoring immune cell activity within the concept of hot and cold fibrosis using appropriate biomarkers could be a game changer for designing new treatments for hot fibrosis. Strategies to increase the infiltration and activation of pro-resolution macrophages in cold fibrosis remain a major challenge for advanced fibrotic diseases.

Further research is warranted to unravel the complex interplay between collagens, the immune system, and fibrosis. We have presented evidence that an organ-specific fibrotic microenvironment can be characterised by non-invasive biomarkers reflecting the deposition and degradation of certain types of collagens and immune cell activity. These markers also have potential prognostic utility and highlight the importance of understanding and characterising different disease phenotypes. Combining biomarkers covering different biological processes represents a potential tool for increased prognostic performance and stratification of phenotypes.

Affiliations

[1]*Nordic Bioscience A/S, Biomarkers & Research, Herlev, Denmark;* [2]*Stravitz-Sanyal Institute for Liver Disease and Metabolic Health and Division of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University School of Medicine, Richmond, VA, USA;* [3]*Translational & Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, UK;* [4]*Newcastle NIHR Biomedical Research Center, Newcastle Upon Tyne Hospitals NHS Trust, Newcastle Upon Tyne, UK;* [5]*Department of Internal Medicine II, Saarland University Medical Centre, Homburg, Germany;* [6]*Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, USA;* [7]*Institute of Translational Immunology and Research Center for Immune Therapy, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany;* [8]*Division of Gastroenterology, Beth Israel Deaconess Medical Center, Boston, Harvard Medical School, MA, USA*

Abbreviations

ALD, alcohol-related liver disease; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CTX-III, cross-linked type III collagen; DDR, discoidin domain receptors; ECM, extracellular matrix; HCC, hepatocellular carcinoma; HNE, human neutrophil elastase; HSCs, hepatic stellate cells; IBD, inflammatory bowel disease; ICIs, immune checkpoint inhibitors; IPF, idiopathic pulmonary fibrosis; LAIR, leukocyte-associated Ig-like receptor-1; KCs, Kupffer cells; LAMs, lipid-associated macrophages; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MMPs, matrix metalloproteinases; MoKCs, macrophages occupying the Kupffer cell; OA, osteoarthritis; PRO-C3, fragment of N-terminal type III collagen; PRO-C6, fragment of C-terminal alpha 3 chain of type VI collagen; RA, rheumatoid arthritis; TGF-β, TGF-transforming growth factor-β; TME, tumour microenvironment; Treg, regulatory T cell.

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Conflict of interest

AZ - full time employee at Nordic Bioscience. DJL – full time employee and stockholder at Nordic Bioscience. AJS – stockholder at Genfit, Rivus, Durect, Tiziana, Northsea, Hemoshear, and Inversago; research grants and consultant: Intercept, Eli Lilly, Novo Nordisk, Boehringer Ingelhiem, Merck, Pfizer, Hanmi, Madrigal, Akero. Histoindex, Avant Sante, Zydus, Pelayo Rubio, Gilead, Resolution Therapeutics, Chemomab, Takeda, Astra Zeneca, Regeneron, Alnylam, Amgen, Hanmi, LG Chem, Path AI, Promed, 89 Bio, Boston Pharmaceuticals, Poxel, Salix, Myovant, Inventiva, Sagimet, Surrozen. QMA – research grants: LITMUS (EU grant agreement number 7773777), AstraZeneca, Boehringer Ingelheim, Intercept and consultant: Alimentiv, Akero, AstraZeneca, Axcella, 89Bio, Boehringer Ingelheim, Bristol Myers Squibb, Galmed, Genfit, Genentech, Gilead, GlaxoSmithKline, Hanmi, HistoIndex, Intercept, Inventiva, Ionis, IQVIA, Janssen, Madrigal, Medpace, Merck, NGMBio, Novartis, Novo Nordisk, PathAI, Pfizer, Pharmanest, Prosciento, Poxel, Resolution Therapeutics, Roche, Ridgeline Therapeutics, RTI, Shionogi, Terns. JMS – stockholder at Hepta Bio; consultant at: Alentis, Alexion, Altimmune, Astra Zeneca, 89Bio, Bionorica, Boehringer Ingelheim, Gilead Sciences, GSK, Ipsen, Inventiva Pharma, Madrigal Pharmaceuticals, Lilly, MSD, Northsea Therapeutics, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi, and Siemens Healthineers. SLF – stockholder at: Galmed, Sunbird Bio, Hepgene, Morphic Therapeutics, Scholar Rock, North Sea Therapeutics, Surrozen, Madrigal; research grant: NIH grant # 5R01DK128289; consultant at Fate Therapeutics. DS – consultancy for Falk Pharma, Ionis, North Sea Therapeutics, Pliant, Prosciento, Resalis, Sanofi, Takeda; DS has received project-related support from the German Research Foundation (DFG) Collaborative Research Center (CRC) grants SFB 1066 project B3, SFB 1292 project B8, and SFB TR355 project B8, LITMUS (EU grant agreement number 7773777). MK – full time employee and stockholder at Nordic Bioscience.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

AZ, DJL and MK conceptualized, wrote and revised the article; AJS, QMA, JMS, SLF, SDS contributed to writing the article and did critical revisions; AZ reviewed and edited the manuscript before submission and was responsible for the figures. All authors approved the final version of the manuscript for submission.

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Supplementary data

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